Assembly of 4.3 yarrowia construct

Restriction

Cut plasmid JMP2563 with BamHI and AvrII (XmaJI)

	Plasmid
Sterile MQ water	Up to 20 µL
10X FastDigest Buffer	2 µL
DNA	Up to 1000 ng
BamHI	1 µL
AvrII (XmaJI)	1 µL
Total	20 µL

30 min 37 degrees (unspecific cutting may occur if incubating longer than 1h with BamHI)

Purify using the PCR purification kit.

Measure concentration.

Prepare Gibson reaction

1. **4.3**

	Cut JME2563	4.3 AQR1	4.3 tXPR2	Mastermix	Sterile MQ water
Conc fragment (ng/µL)	9,9	29	26,6	X	X
Volume (µL)	6	2,5	1,5	10	0

2. **Positive control**
   Prepare positive control: 10 µL of control + 10 µL of Gibson mastermix

Gibson reaction
50 degrees 2 hour

Transform to *E. coli* with standard heat shock protocol (4 tubes of ecoli).

- Transforms 5 µL of 4.3 Gibson assembly to E. coli
- Also transform 2 µL of Gibson assembly, both 4.3 and positive control
- Do a negative control: Transform 1,5 µL of cut JME2563 (15 ng: (60/ 20) *5) to *E. coli*
- Plate 4.3 on LB + kanamycin
- Plate positive control on LB + amp
- Plate negative control on LB + kanamycin

- 37 degrees’ overnight

Replate

- Restreak 4.3 colonies + incubate in LB+kanamycin overnight 37 degrees

Miniprep

Follow protocol. Spin longer with the column empty to remove ethanol. Elute with 50 µL elution buffer.

Restriction verify with XbaI and SpeI (BcuI)

	Plasmid
Sterile MQ water	Up to 10 µL
10X FastDigest Green Buffer	1 µL
Construct 4.3	150 ng
XbaI	0.5 µL
SpeI (BcuI)	0.5 µL
Total	10 µL

60 min 37 degrees

Load 10 µL on gel. Post stain with GelRed.

Should look like **lane 1:**

MW: GeneRuler™ 1 kb DNA Ladder

1: Assembled AQR1 + tXPR2 in JME2563

SpeI + XbaI

1. 5505 bp

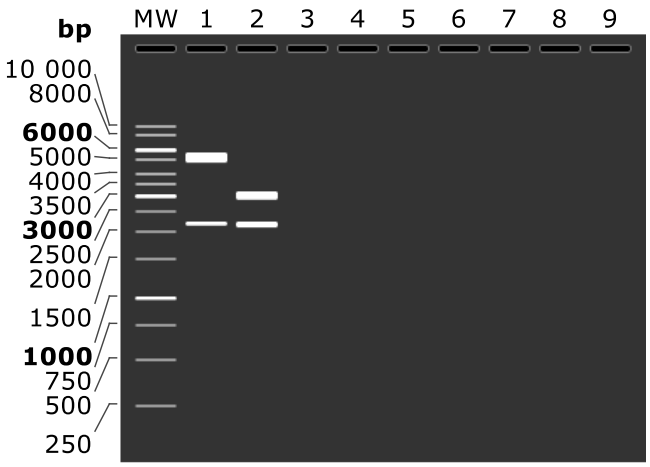
2. 2200 bp

2: Plasmid_yarrowia_2_JME2563_JMP62-LEU2Ex-pTEF

XbaI + SpeI

1. 3161 bp

2. 2200 bp



Transform to yeast by electroporation

Colony PCR

Genomic extraction of isolated colonies. **Use extension time of 72 seconds (1:12) for both**

1. Use primers: AQR1 F (IS) and tXPR2 (IS)
   Anneal at 65 degrees
   Product should be 2390 bp
2. Positive control:
   Use primers: Gln1 F (JMP1047) and Gln1 R (tXPR2)
   Anneal at 61.
   Product should be 1408 bp